Analysis of Pancreas Histological Images for Glucose Intolerance Identification using ImageJ – preliminary results

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ABSTRACT

The observation in microscopy of histological sections allows us to evaluate structural differences, in pancreatic cells, between rats with normal glucose tolerance and with glucose intolerance (pre-diabetic) situation. Nevertheless, this pre-diabetic condition implies subtle changes in islets of Langerhans structure. This and the normal variability among sampled cells makes difficult the task of identifying glucose intolerance (pre-diabetic situation) with a low level of error. This paper presents preliminary results in the processing of histological pancreas images with the goal of identifying pre-diabetic situation in Wistar rats. The immediate goal of this work is to evaluate the performance of a classifier based in a morphometric measurement of the histological images and to assess the potential for image based automatic processing and classification. A set of 90 images, were used (58 from rats with normal glucose tolerance, and 32 from pre-diabetic ones). These images were segmented manually using ImageJ. This segmentation and area measurements have been speedup by the application of ImageJ macros which were defined for this purpose. The ratio, between the area of β-cells and the islets of Langerhans , was used has the indicator of the pre-diabetic situation. Considering this feature, a receiver operating characteristic analysis has been performed. True positive rate, vs. false positive rate shows the predicted performance of a binary classifier as its discrimination threshold is varied.

1 INTRODUCTION

The evaluation of histological images made through quantitative criteria using computational tools and algorithms allows to speedup analysis process and attain more accurate results[1, 2, 3]. The immediate goal of the presented work is to evaluate the performance of a OneRule classifier based in the ratio between the measured region with β-cells and the islets of Langerhans, and thus to assess the potential precision, specificity, and the sensitivity of a diagnostic test, based on such a ratio. Morphometric analysis of islets of Langerhans have been studied and has been demonstrated to be relevant to discriminate glucose intolerance[4]. Moreover, other structural differences are notorious, in a full diabetic situation[5]. In the following, in this paper it is described the set of used images; the definition of the regions of interest (ROI) in each image; presentation of results of the extracted feature; presentation of a ROC analysis; conclusions and ongoing work towards an automatic image processing and classification.

2 IMAGE SAMPLES

Wistar rats have been selected for their glucose intolerance (GIR). In this work GIR females were compared with standard Wistar females with normal glucose tolerance (control). From an original set with approximately 300 images, we used 90 pancreas photomicrographs (with magnification 100X) of Wistar rats belonging to two groups: Group I (normal glu-

cose tolerance) with 58 samples, Group II (glucose intolerant/pre-diabetic) with 32 samples.

Pancreata from control (Wistar) and glucose intolerant rats (GIR) were excised, fixed and prepared for immunohistochemistry analysis of insulin positive cells[4].

From the original the selection of 90 images was made, eliminating every image with a ROI - the islet of Langerhans - considered too small. This limit was defined considering only images were the selected pancreatic islet area was larger than 90.10^3 pixels. Figure 1 illustrates one such image.

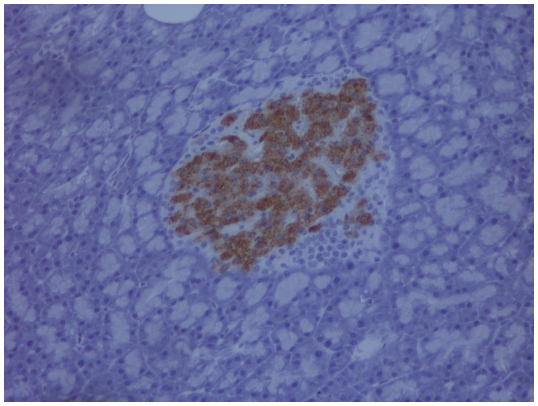

Figure 1: Histological image of pancreas with islets of Langerhans and beta cells (brown color).

3 IMAGE PROCESSING

The image processing and manipulation is based in ImageJ software[6]. ImageJ is a Java based free software application for image processing developed at the United States National Institutes of Health (NIH). ImageJ was designed providing extensibility via Java plugins and recordable macros. Being a Java-based application ImageJ runs easily in any Windows, MacOS, Linux, ou Unix-like systems, with a java installation. ImageJ makes possible to define Macros that are important to speed up routine repetitive tasks. In this work booth developed Macros are activated through keyboard shortcuts.

The 90 selected images were processed in the following way:

- Manual definition of ROI 1 - islet of Langerhans.
- Macro 1 activation - measurement of ROI 1.
- Manual definition of the area of β-cells - ROI 2.
- Macro 2 activation - Definition of ROI 3 (ROI 1 subtracted by ROI 2); measurement of ROI 3; calculation of the ratio between ROI 3 and ROI 1; and results are saved in a spredsheet file $Results.xls$

An example with the sequence of steps with the delimitation of ROIs(Regions of interest) is presented in Figures 2, 3, and 4.

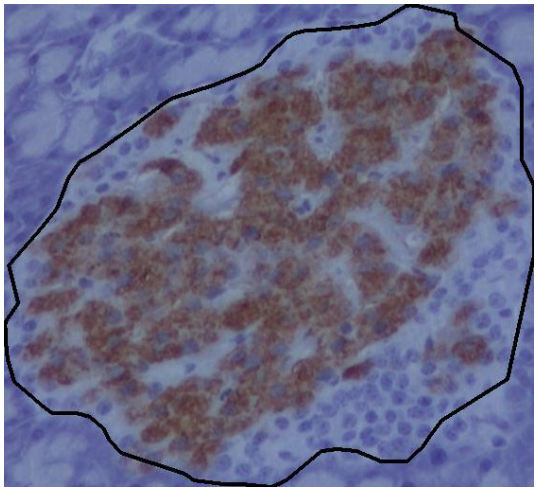

Figure 2: Histological image of pancreas cells islets of Langerhans and beta cells (brown color) manually defined region - islet of Langerhans.

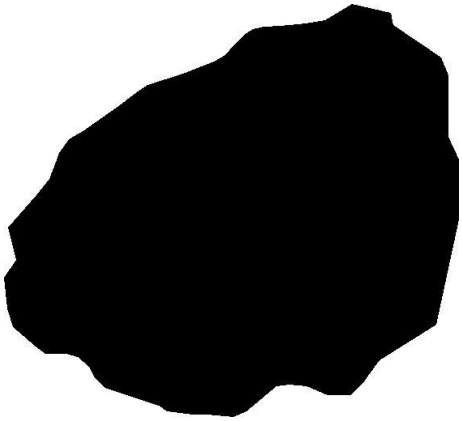

Figure 3: Region of Interest 1.

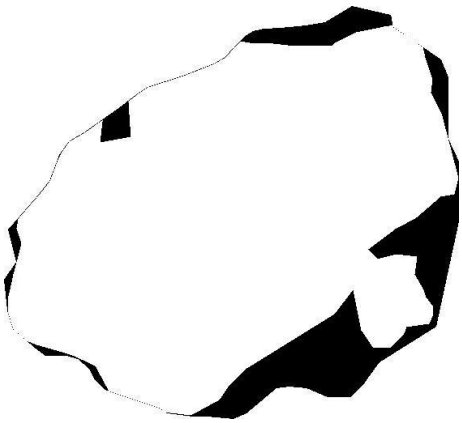

Figure 4: ROI 3:Region of interest 1 subtracted by Region of interest 2.

4 RESULTS

From the morphometric measurement of the 90 images, it is possible to observe that the average of area ratio - between ROI 3 and ROI 1 - are for the control and GIR group, 0.103 and 0.213, respectively. Histograms with the relative frequency of this area ratio, for both groups, are presented in Figure.5. This difference is according to previous studies [4, 5]. The variance observed in the histograms, is also according to structural variance inside each pancreas.

From the above calculations a ROC curve has been drawn, see Figure6. This ROC curve illustrates the

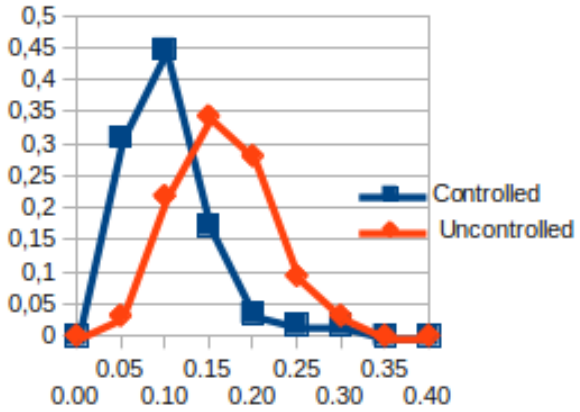

Figure 5: Histogram - relative frequency of the area ratio between non-beta cells and all cells.

compromise between False Negative and False Positive Errors.

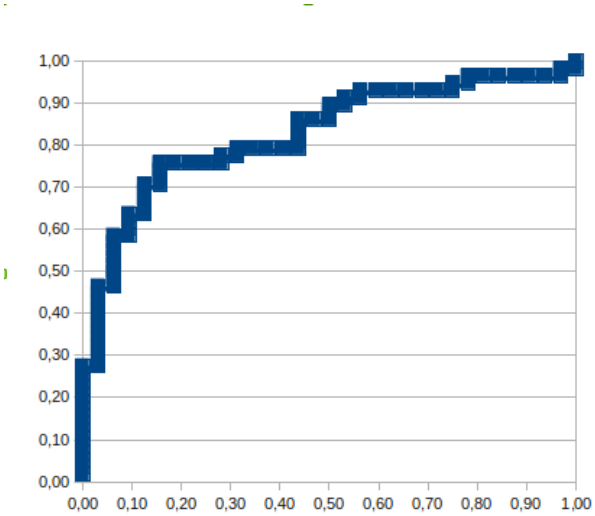

Figure 6: ROC curve – True Positives rate vs. False Positives rate.

5 CONCLUSIONS AND ONGOING WORK

The paper presents preliminary results in the processing of histological pancreas images identifying pre-diabetic situation in rats. The results have been presented through a receiver operating characteristic curve that shows promising results for a classification task. The obtained results should be considered twofold. Firstly, this should be considered as a goal line to an automatic segmentation process, to identify islet of Langerhans area and the β-cells inside it. Secondly, considering that other works have identified clearly modified (e.g. fragmented) structure in islet of Langerhans in pancreas with a full diabetic situation[5], there are other features that once extracted can potentially increase the precision of an automatic classifier. Ongoing work pursues this goal through fractal analysis of ROIs; perimeter per area ratio; and texture identification inside the islet of Langerhans.

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